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L4: Entry 10 of 51

File: JPAB

May 12, 1998

PUB-NO: JP410120589A

DOCUMENT-IDENTIFIER: JP 10120589 A

TITLE: ANTIBACTERIAL AGENT AND ANTIBACTERIAL FOOD

UBN-DATE: May 12, 1998

INVENTOR-INFORMATION:

NAME

COUNTRY

SAKUMA, KAZUO

ASSIGNEE-INFORMATION:

NAME

COUNTRY

SAKUMA KAZUO

PPL-NO: JP08297713

PPL-DATE: October 21, 1996

INT-CL (IPC): A61 K 35/84; A23 L 3/3571

ABSTRACT:

PROBLEM TO BE SOLVED: To obtain the subject agent and food capable of destroying or suppressing pathogenic bacteria and suppressing the development of a disease caused by the bacteria by including an extract of Fuscoporia obliqua.

SOLUTION: This antibacterial agent consists of an extract of Fuscoporia obliqua. The natural and cultured F. obliqua are both effective, but since the natural ones are hard to collect and the yield is small, it is preferable to use the artificially cultured ones. It is preferable to use a new dust culturing and a liquid culturing method. The extract of the F. obliqua having suitably 10000-1500000, more suitably 10000-100000 molecular weight, has the antibacterial activity. Thus, it can effectively be used as a medicine or by mixing with a food against the pathogenic bacteria e.g. pathogenic E. coli, pneumobacillus and Pseudomonas aeruginosa).

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L4: Entry 45 of 51

File: DWPI

May 12, 1998

DERWENT-ACC-NO: 1998-328404

DERWENT-WEEK: 199829

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TITLE: Use of Fuscoporia obliqua Aoshima extract - as antibacterial agent and in antibacterial foods.

PATENT-ASSIGNEE:

ASSIGNEE

CODE

SAKUMA K

SAKUI

PRIORITY-DATA: 1996JP-0297713 (October 21, 1996)

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PATENT-FAMILY:

PUB-NO

PUB-DATE

LANGUAGE

PAGES

MAIN-IPC

☐ JP 10120589 A

May 12, 1998

005

A61K035/84

APPLICATION-DATA:

PUB-NO

APPL-DATE

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DESCRIPTOR

JP 10120589A

October 21, 1996

1996JP-0297713

NT-CL (IPC): A23 L 3/3571; A61 K 35/84

ABSTRACTED-PUB-NO: JP 10120589A

BASIC-ABSTRACT:

An antibacterial agent comprises extract from natural and cultured Fuscoporia obliqua Aoshima.

Fuscoporia obliqua Aoshima is preferably cultured in sawdust medium and extracted with hot water to give a powder or liquid extract of molecular weight of 10000-1500000 (preferably 10000-100000). The pathogenic bacteria are Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Clostridium perfringens.

ADVANTAGE - The extract can be formulated as healthy foods with antibacterial effects.

DRAWING-DRAWING: Dwg.0/0

TITLE-TERMS: EXTRACT ANTIBACTERIAL AGENT ANTIBACTERIAL FOOD

DERWENT-CLASS: B04 D13

PI-CODES: B04-A10; B14-A01; D03-H01;

CHEMICAL-CODES:

Chemical Indexing M1 \*01\*

Fragmentation Code

M423 M710 M903 P220 Q211 V500 V550

SECONDARY-ACC-NO:

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A 2 3 L 3/3571		A 2 3 L 3/3571

審査請求 未請求 請求項の数11 F D (全 5 頁)

(21) 出願番号	特願平8-297713	(71) 出願人	592142496 佐久間 和夫 北海道上川郡下川町上名寄2119の1
(22) 出願日	平成8年(1996)10月21日	(72) 発明者	佐久間 和夫 北海道上川郡下川町上名寄2119の1

(54) 【発明の名称】 抗菌剤及び抗菌性食品

(57) 【要約】 (修正有)

【課題】カバノアナタケの抽出物を利用して病原性細菌を死滅させ又は抑制し、細菌性疾病の発現を抑制する技術を提供する。

【解決手段】カバノアナタケの抽出物を有効成分として、これをそのまま又は他の物質を加えて服用することにより、病原性細菌、特に病原性大腸菌O-157、及びその他の細菌の発育を阻止することができる抗菌剤及び抗菌性食品が得られる。病原性細菌としては、病原性大腸菌のほか、肺炎桿菌、緑膿菌、黄色ブドウ球菌及びガス壊疽菌などにも本発明の抗菌剤及び抗菌性食品は有効である。

## 【特許請求の範囲】

【請求項1】 カバノアナタケの抽出物から成り、病原性細菌の発育を阻止する作用がある抗菌剤。

【請求項2】 カバノアナタケが天然産である請求項1に記載の抗菌剤。

【請求項3】 カバノアナタケが人工培養物である請求項1記載の抗菌剤。

【請求項4】 カバノアナタケがオガクズ培地で培養されたものである請求項3に記載の抗菌剤。

【請求項5】 カバノアナタケの抽出物がオガクズ培地で培養されたものを熱水抽出した粉末又は液である請求項4に記載の抗菌剤。

【請求項6】 カバノアナタケ抽出物が分子量10,000~1,500,000の範囲である請求項5に記載の抗菌剤。

【請求項7】 カバノアナタケ抽出物が分子量10,000~100,000の範囲である請求項6に記載の抗菌剤。

【請求項8】 病原性細菌が、病原性大腸菌、肺炎桿菌、緑膿菌、黄色ブドウ球菌及びガス壊疽菌から成る群から選ばれた細菌である請求項1に記載の抗菌剤。

【請求項9】 カバノアナタケの抽出物がオガクズ培地で培養されたものを熱水抽出した分子量10,000~1,500,000の範囲のものであり、病原性細菌が、病原性大腸菌、肺炎桿菌、緑膿菌、黄色ブドウ球菌、ガス壊疽菌から成る群から選ばれた細菌である請求項8に記載の抗菌剤。

【請求項10】 カバノアナタケの抽出物を食品、飲料、調味料等に加えて混合して成り、病原性細菌に対する発育阻止作用を保持した抗菌性食品。

【請求項11】 カバノアナタケが天然産又は人工培養物であり、その抽出物を粉末又は溶液の形で、醤油、味噌、塩などの調味料；パン、ケーキ、アイスクリーム、チョコレート、冷凍、せんべい、鮎、ゼリー、グミ等々の菓子類；うどん、冷麦、スパゲティなど食品類；ソーセージ、ハンバーグ、コロッケ、てんぷら、かまぼこ、調味肉等々の肉加工品；ビール、ワイン、焼酎などのアルコール類又はアルコール飲料、にんにくエキス、トマト、人参、野菜ミックスジュース、りんご、グレープ等々の果実ジュース、炭酸飲料；紅茶、ウーロン茶、ヤンロン茶等の発酵茶；コーヒー、緑茶、ココア飲料に混合して成る抗菌性食品。

## 【発明の詳細な説明】

## 【0001】

【発明の技術分野】 本発明は、菌類起源の抗菌剤に関するもので、具体的にキノコ、特にカバノアナタケの抽出物を活性成分とする病原性細菌に対する抗菌剤と、この抗菌活性成分を含有する抗菌性食品に関するものである。

## 【0002】

【発明の背景】 種々の細菌が人間を取り巻いて、特に病原性細菌との闘いは人間の永遠のテーマともいえる。通常、病原性細菌に対しては抗生物質が使用され、それなりの効果を上げてきたが、そのたび重なる使用の結果、耐性菌が増加して新たな問題を投げかけている。例えば大腸菌は常在菌であり、一般には病原性がないとされているが、なかには近ごろ問題になった大腸菌O-157のように激しい病原性を示す大腸菌もあり、在来の治療薬では著効がないようなものも出てきた。こうした状況の中で、在来の抗生物質に代わる、或いはそれと協働する新たな抗菌剤や健康食品の出現が望まれている。

【0003】 本発明者は、これまでカバノアナタケ〔学名 *Fuscospora obliqua* (Fr.) Aoshima〕の抽出物が抗ガン作用を有すること、及び抗エイズ作用を有することを見出し、特許出願をしている（特願平1-114665 服用可能な抗ガン剤と食品及び菌糸体製造方法及び特願平8-23208 抗HIV剤）。これらの知見をもとに、さらに研究を重ねたところカバノアナタケの抽出物にはLPS (Lipopolysaccharide) すなわちリポ多糖体が非常に多く含有されていることが確認された。そこから、カバノアナタケ抽出物はガンやHIVだけではなく、病原性細菌、特にO-157などのような腸内細菌にも効果があるとの見通しを得たので、その縁に沿って研究を重ね、この発明を成立させるに至ったものである。

## 【0004】

【発明が解決しようとする課題】 本発明は、カバノアナタケの抽出物を利用して病原性細菌を死滅させ又は抑制し、細菌性疾患の発現を抑止する技術を提供することとする。

## 【0005】

【課題を解決するための手段】 本発明は、カバノアナタケの菌糸の抽出物を有効成分としてO-157などの腸内菌及びその他の病原性細菌を不活性化する抗菌剤と、同有効成分を含有する健康（抗菌性）食品を作るものである。カバノアナタケは、真菌類の中の担子菌亜門、帽菌亜綱、ヒダナシタケ目、タバコウロコタケ科、サビアナタケ属、カバノアナタケ種〔学名 *Fuscospora obliqua* (Fr.) Aoshima〕と分類されているもので天然にはシラカバ、ダケカンバなどのようなカバノキ類の立木の幹に生育し、石炭の集塊のような黒色の菌核を形成する。菌核は径20cmにもなることがあり、元来はシラカバなどのカバノキ類にとって有害な菌（いわばガン）として知られていた。しかしながら本発明者は、カバノアナタケ（俗称チャガ）が強い生命力をもつことに着目して研究を開始し、その菌糸の抽出物が抗ガン作用及びエイズウイルスの増殖抑制作用を有することを見出して前記のように特許出願をした。本発明は、これらの知見を重ねた新たな研究を行い、カバノアナタケ抽出物が病原性大腸菌、特にO-157や、その他肺炎菌、緑膿菌、黄色ブ

ドウ球菌、ガス壊疽菌などの抑制乃至発育阻止作用があることを見出したものである。

【0006】本発明においてカバノアナタケは天然産も培養物も同等に有効であるが、天然カバノアナタケは採取が容易ではなく、また収量も少ないので、大量に必要とする場合は人工培養法により得られるカバノアナタケを利用するのが好適である。人工培養は、いくつかの方法があるが、好適にオガクズ培養及び液体培養によること  
10 ことができる。そのほか、人工的にカバノキ類の生木にカバノアナタケ菌糸を植菌して増殖させた菌糸、又はこの菌糸を生育させて形成させた菌核も本発明において有効に利用することができる。人工植菌したものは天然産に準じる。

【0007】アバノアナタケの人工培養法の1つ、オガクズ培養については、すでに先の特許出願（特開平1-114665号）に記載してあるが、ここに要説すると以下の通りである。まず、シラカンバ又はダケカンバなどのカバノキ類の樹木の木片（オガクズ）を用意し、これに石灰、米ヌカを入れ、水分を調整してきた混合物を耐熱性の容器又は袋に詰め、これを加熱滅菌してオガクズ培地とする。このオガクズ培地をカバノアナタケ菌糸の生育温度、好適には20℃以下に冷却したのち、この培地にカバノアナタケ純粋菌糸を植菌してカバノアナタケ菌糸体を生育させる。長期間、好適には菌核状（黒色）のものできるまで生育させてから収穫し、必要な  
20 処理により有効成分を抽出し、抗菌剤又は食品として利用する。

【0008】カバノアナタケの有効成分を抽出するには種々の方法があるが、例えば、天然カバノアナタケまたは培養カバノアナタケの菌糸をPBS溶液、ブタノール、エタノール、酢酸エチルまたはアセトンにより処理し、その各不溶物から活性成分を取り出すことができる。或いは、天然カバノアナタケまたは培養カバノアナタケの菌糸成分または菌糸抽出物をカーボンまたはチャコールで吸着処理し、その各非吸着物から活性成分を取り出すこともできる。また、培養カバノアナタケまたは培養カバノアナタケを種々なpHの水で煮沸（例えば6\*  
30

\*0分間煮沸）する熱水抽出によっても有効成分を得ることができる。本発明によるこれら抽出活性成分は、水溶性で耐熱性、耐酸性ある安定な物質であると認められる。これら抽出成分は、そのまま、或いは食品類に混合して経口摂取することができる。

【0009】本発明において、カバノアナタケ抽出物の抗菌作用は病原性細菌、特に下記の表1に示す種々の細菌すなわち大腸菌O-157、肺炎桿菌、緑膿菌、黄色ブドウ球菌、枯草菌、ガス壊疽菌について、最小発育阻止濃度(MIC)測定法（日本化学療法学会）にしたがって試験を行なうことにより確認された。各菌株を、ガス壊疽菌以外は、感受性ディスク用培地に所定菌数（表1の接種菌数）を接種し、またガス壊疽菌は0.1%チオグリコール酸ナトリウム加ブレンハートインフュージョン寒天培地に接種して上記測定法により試験した。

【0010】本発明において抗菌活性試験に使用するカバノアナタケ抽出物の生成法の一例を述べると次の通りである。まず、粉碎したカバノアナタケ（天然産又は人工培養物）の粉末、例えば100gを蒸留水1000ccに入れ、100℃で40分間煮沸することにより熱水抽出する。ついで、ガーゼ又は濾紙を用いて抽出液を濾過し、さらに遠心分離を行なう。遠心分離は、例えば3000rpmの遠心分離機で約10分間行なう。ついで、再び濾過し、凍結乾燥して、目的とするチャグ抽出物有効成分を得る。こうして得られるカバノアナタケ抽出物は、或る分子量以上で抗菌作用があるものと認められる。例えば、熱水抽出物そのままで、まだ分画していないものは、おむね分子量10万台以上に顕著な抗菌活性が認められるが、分画した場合、熱水抽出で活性成分は特に分子量3万以上に  
30 強い活性が認められた。広い範囲では分子量10,000~5,000, 000まで活性が認められるが、好適に分子量10,000~1,500,000, より好適に10,000~100,000の範囲に抗菌活性があると認められる。本抗菌活性試験の結果を表1に示す。

【0011】

【表1】

病原性細菌の種類	接種菌数	最小発育阻止濃度(ppm)
E. coli O-157(EDL 931) (大腸菌)	2.7×10 <sup>7</sup>	10,000
K. pneumoniae (ATCC 4352) (肺炎桿菌)	8.0×10 <sup>6</sup>	10,000
P. aeruginosa (IFO 13276) (緑膿菌)	2.8×10 <sup>6</sup>	5,000
S. aureus (IFO 12732) (黄色ブドウ球菌)	2.2×10 <sup>7</sup>	5,000
B. subtilis spore (ATCC 6633) (枯草菌)	1.4×10 <sup>6</sup>	100,000
C. perfringens H2 (ガス壊疽菌)	2.0×10 <sup>6</sup>	2,600

【0012】抗菌活性はカバノアナタケに特有なものか、それとも他のキノコにも抗菌作用があるのかを確認するため、次の試験を行った。候補のキノコは、カバノアナタケのほか、マイタケ (*Grifola frondosa* (Dicks: F r))、シイタケ (*Lenzites edodes* (Berk) Sing.) である。これら候補キノコの生の材料を各100gずつ取って容器に入れ、これに蒸留水1000ccを加えて、100℃で40 \*

試料	菌種	接種菌数	最小発育阻止濃度
(1)	<i>E. coli</i> 0157 (EDL 931)	$2.7 \times 10^7$	10,000ppm
(2)	<i>E. coli</i> 0157 (EDL 931)	$1.0 \times 10^6$	阻止できず
(3)	<i>E. coli</i> 0157 (EDL 931)	$1.0 \times 10^6$	阻止できず

試料(1)=カバノアナタケ熱水抽出物

試料(2)=マイタケ

試料(3)=シイタケ

【0014】カバノアナタケが他のキノコにない抗菌作用を有する根拠は現在のところ明確には把握されていないが、人体を細菌から守る生体活性の1つは免疫増強作用である。免疫に関与する細胞にはリンパ球とマクロファージとがあり、マクロファージの活性化にはLPS (リボ多糖体) が寄与している。本発明者が着目したカバノアナタケがこのような有効物質をどの程度含有しているかを確認するため試験を行ったので、その結果を表3に示す。検体は、カバノアナタケのほか、ニンジンエキスを、ニンジン葉エキスをを用いた。検体の調製は、カバノ

検体名	LPS含有量( $\mu\text{g/g}$ )
カバノアナタケエキス	1,610
ニンジンエキス	0.85
ニンジン葉エキス	29.5

【0016】以上のことから、本発明によるカバノアナタケの抽出物が、病原性細菌、特に最近非常に問題になっているO-157に対し有効な抗菌作用を有することが判明した。本発明のカバノアナタケ抽出物は、薬剤として粉末又は液体例えば水溶液の形で経口摂取し、有害な病原性細菌を死滅させ乃至発育を阻止することができる。また同抽出物は食品類に混合添加することにより健康食品として摂取することもできる。例えば、本発明のカバノアナタケ抽出物は、粉末、溶液又はその他の形で醤油、味噌、塩などの調味料に混合して食用とすることができる。またパン、ケーキ、アイスクリーム、チョコレート、冷凍、煎餅、鮎、ゼリー、グミ等の菓子類に、或いはうどん、冷茶、スバゲティなどの加工食品類に、製造時にカバノアナタケ粉末又は溶液を添加混合して健康食品とすることができる。同様に、ソーセージ、ハンバーグ、コロッケ、てんぷら、かまぼこ、調味肉等々の肉製品に製造時に混合してもよいし、ビール、ワイン、焼酎などのアルコール類又はアルコール飲料や、にんにくエキス、トマト、人参、野菜ミックスジュース、りんご、グレープ等々の果実ジュース、炭酸飲料など、或いはさらに紅茶、ウーロン茶、ヤンロン茶等の発酵茶やコーヒー、緑茶、ココア飲料などに製造時又は★50

\* 分間煮出して抽出液を出す。この抽出液を東洋口紙 No. A5 を用いて濾過すると、各候補のエキス試験液200cc が得られた。このエキスをを用いて、前記と同様感受性ディスク用培地を用い抗菌テストを行った。結果を表2に示す。

【0013】

【表2】

※ノアナタケ、ニンジンエキス、ニンジン葉エキスのそれぞれ500 mg を精秤し、これらに注射用蒸留水を加えて終濃度100mg/ml とした。これを56℃で3時間水浴中で加温抽出した後、遠心分離し、遠心上清を取り、これを測定用検体の原液とした。LPS の定量方法は、LPS を特異的に測定できるLimulus amoebocytolysate assay の比色定量法 (生化学工業) により行った。

【0015】

【表3】

★ 飲用時に混合して健康飲料とすることもできる。以上、例示した調味料、菓子類、加工食品類、肉製品、飲料類にカバノアナタケ抽出物を添加したものを本発明においては「健康食品」又は「抗菌性食品」と総称する。【0017】カバノアナタケの人工培養法は、前記のようにすでに別出願で完成されているが、そのうち特にオガクシ培養物の熱水抽出物の粉末体には、ほろ苦く、香ばしいという特性があるため、これをビールやその他の飲料に添加することにより、病原性細菌の発育抑制作用のほか、に、苦みやコクを付加し、また甘み原料としてのホップの代用とする用途も開発される。また、カバノアナタケ特有の黒い色素を活用することで、醤油製造時のカラメルの添加に代えることができ、その他安全かつ健康保持に適した食用色素としてカバノアナタケ黒色粉末を用いることもできる。例えば、カバノアナタケの黒色粉末を利用して健康保持作用をもった黒いマンジュウを作るとか、アズキあんのに色の深みを出すとか、また人体の健康上に何等有害な作用がないから布の染料として、或いは白染めにも応用することができる。さらに、カバノアナタケの抽出物又はそのエキスをたばこ製造時にたばこ葉に混入することにより、喫煙者が無視なく抗菌性物質を摂取できるようにすることもできる。

## 【0018】

【発明の効果】以上説明の通り、本発明によればカバノアナタケの抽出物を、直接的に抗菌剤として経口服用することにより、又は同抽出物を添加混合した健康食品又は抗菌性食品を常時摂取することにより、病原性細菌、特に近時異常な蔓延を見せている恐ろしい病原性細菌O-157を死滅させ又は発育阻止する作用が期待される。本発明のカバノアナタケ抽出物は、長年の研究によ

り人体に対する無害性が確認されているから、継続服用しても健康上問題を生じることはなく、従来多用されてきた抗生物質のような耐性菌の発生のおそれもない。O-157に限らず、病原性細菌に起因する種々の疾病に対し、常時、広く本発明の抗菌剤又は抗菌性食品を使用することにより、健康を保持し、細菌性疾病を予防する著大な実用効果が得られる。



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**ANTIBACTERIAL AGENT AND ANTIMICROBIAL FOODSTUFF**  
[抗菌剤及び抗菌性食品]

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(57) [Abstract]

(There is an amendment.)

[Problems to be Solved by the Invention]

extermination doing pathogenicity bacterium making use of extract of *Fuscoporia obliqua*, or you control, you offer technology which controls revelation of the bacterial disease.

[Means to Solve the Problems]

With extract of *Fuscoporia obliqua* as active ingredient, this pathogenicity bacterium, especially pathogenicity *E. coli* O-157, and antibacterial agent and antimicrobial foodstuff which can obstruct growth of other bacterium are acquired by administration doing that way or including other substance.

As pathogenicity bacterium, other than pathogenicity *E. coli*, antibacterial agent and antimicrobial foodstuff of the this invention are effective even in *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and gas gangrene microbe, etc.

[Claim(s)]

[Claim 1]

antibacterial agent. which is action which consists of extract of *Fuscoporia obliqua*, obstructs growth of pathogenicity bacterium

[Claim 2]

antibacterial agent. which is stated in Claim 1 where *Fuscoporia obliqua* is natural product

[Claim 3]

antibacterial agent. which is stated in Claim 1 where *Fuscoporia obliqua* is artificial culture

[Claim 4]

*Fuscoporia obliqua* being sawdust culture medium, culture antibacterial agent. which is stated in the Claim 3 which is something which is done

[Claim 5]

extract of *Fuscoporia obliqua* being sawdust culture medium, culture antibacterial agent. which is stated in Claim 4 which is a powder or a liquid which those which are done hot water extraction is done

[Claim 6]

antibacterial agent. which is stated in Claim 5 where *Fuscoporia obliqua* extract is range of molecular weight 10, 000-1, 500, 000

[Claim 7]

antibacterial agent. which is stated in Claim 6 where *Fuscoporia obliqua* extract is range of molecular weight 10, 000-100, 000

[Claim 8]

pathogenicity bacterium, antibacterial agent. which is stated in Claim 1 which is a bacterium which is chosen from group which consists of pathogenicity *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and the gas necrosis microbe

[Claim 9]

extract of *Fuscoporia obliqua* being sawdust culture medium, culture with those of range of molecular weight 10, 000-1, 500, 000 which those which are done hot water extraction is done, pathogenicity bacterium, the antibacterial agent. which is stated in Claim 8 which is a bacterium which is chosen from group which consists of pathogenicity *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, gas necrosis microbe

[Claim 10]

adding and mixing doing extract of *Fuscoporia obliqua* in foodstuff, beverage, flavoring, etc antimicrobial foodstuff. which becomes, keeps growth inhibition action for pathogenicity bacterium

[Claim 11]

*Fuscoporia obliqua* with natural product or artificial culture, in form of powder or the solution, to meat processed good; beer, wine, distilled rice spirits or other alcohols of foodstuffs; sausage, hamburger, croquet, tempura, fish past cake, flavoring meat, etc., or fruit juice, carbonated beverage; black tea, oolong tea, yanron tea or other fermentation tea; coffee, green tea, cocoa beverage of the alcohol beverage, garlic extract, tomato, ginseng, vegetable mix juice, apple, grape etc. such as

soy sauce, miso paste, salt or other flavoring; pan, cake, ice cream, chocolate, frozen confection, rice crackers, candy and confection of jelly, oleaster, etc.; udon noodle, cold cereal grain, spaghetti mixing extract antimicrobial foodstuff. which becomes

[Description of the Invention]

[0001]

[Technological Field of Invention]

this invention being something regarding antibacterial agent of fungi origin, is something regarding antimicrobial foodstuff which contains antibacterial agent and this antibiotic activity component for pathogenicity bacterium which concretely designates extract of mushroom, especially *Fuscoporia obliqua* as active ingredient.

[0002]

[Prior Art]

various bacterium having surrounded person, even eternal theme of the person you can call dispute with especially pathogenicity bacterium.

Usually, antibiotic was used with respect to pathogenicity bacterium, increased effect of that appearance, but result of use which that each time is piled up, resistant fungi increasing, new problem is thrown.

for example *E. coli* is assumed that with normally present microbe, there is not a pathogenicity generally, but in like *E. coli* O-157 which becomes recent problem there will be also a *E. coli* which shows extreme pathogenicity, with common therapeutic work effectiveness will be thing came out.

In such status, it substitutes to common antibiotic, or that and appearance of new antibacterial agent and health food which cooperation working are done is desired.

[0003]

As for this inventor, extract of *Fuscoporia obliqua* {scientific name *Fuscopia obliqua* (Fr.) Aoshima } has anticancer effect so far, and the fact that it possesses anti-AIDS action is

discovered, patent application is done, (Japan Patent Application Hei 1- 114665 "administration possible anticancer agent and foodstuff and mycelia manufacturing method " and Japan Patent Application Hei 8-23208 "anti-HIV drug " ).

These knowledge when furthermore research is repeated on basis of LPS (lipopolysaccharide) namely lipopolysaccharide non- normally mainly being contained was verified in extract of *Fuscoporia obliqua*.

From there, *Fuscoporia obliqua* extract not only cancer and HIV, when there is a effectiveness even in pathogenicity bacterium, especially O-157 or other intestinal bacteria, because forecast was acquired, research is repeated alongside that line, being formed reaches point of this invention it is something which.

[0004]

[Problems to be Solved by the Invention]

extermination doing pathogenicity bacterium making use of extract of *Fuscoporia obliqua* or you control this invention, you designate that technology which controls revelation of bacterial disease is offered as problem.

[0005]

[Means to Solve the Problems]

this invention is something which makes health (antimicrobial) foodstuff which contains antibacterial agent and same active ingredient which microbe and other pathogenicity bacterium inside O-157 or other intestine inactivation are done with extract of the mycelia of *Fuscoporia obliqua* as active ingredient.

Basidiomycetes subphylum in Eumycota, hat microbe sub- tow, crimp pear bamboo eye, *Nicotiana tabacum* L. (tobacco) fish scales bamboo course, *Fuscoporia* being attached, *Fuscoporia obliqua* kind {scientific name *Fuscoporia obliqua* (Fr.) Aoshima } with being something which classification is done, in natural *Betula tauschii* (white birch), you grow *Fuscoporia obliqua*, in the trunk of stands of *Betula ermanii* or other Betulaceae, you form sclerotium of black like cluster of coal.

As for sclerotium there was a thing which becomes also diameter 20 cm, originally it was known toxic microbe (In a manner of speaking cancer) as for *Betula tauschii* (white birch) or other

Betulaceae.

But this inventor paying attention to having life power where *Fuscoporia obliqua* (common name chaga) is strong starts research, discovering fact that extract of mycelia has proliferation-suppressing action of anticancer effect and AIDS virus the aforementioned way it did patent application.

this invention researches, repeating to these knowledge, new, *Fuscoporia obliqua* extract pathogenicity *E. coli*, especially O-157 and, in addition is something which discovers fact that it is pneumonia microbe, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, gas necrosis microbe or other control to growth inhibition action.

[0006]

Regarding to this invention, *Fuscoporia obliqua* natural product and culture and is effective equally, but natural *Fuscoporia obliqua* recovery not to be easy, in addition because yield are little, when it needs for large scale, it is ideal to utilize *Fuscoporia obliqua* which is acquired by artificial culturing.

artificial culture are several method, but it can do thing ideally with the sawdust culture and liquid culture.

In addition, in artificial inoculation doing *Fuscoporia obliqua* mycelia in greenwood of Betulaceae, growing mycelia, or this mycelia which multiplied, also sclerotium which it formed regarding to this invention, it can utilize effectively.

Those which artificial inoculation are done correspond to natural product.

[0007]

Concerning one, sawdust culture of artificial culturing of *Fuscoporia*, it is already stated in patent application (Japan Patent Application Hei 1- 114665 number) ahead, but when main point theory it does here, as follows is.

First, you prepare *Betula platyphylla* Sukatscher var. *japonoca* (Miq.) Hara or wood chip (sawdust) of tree of *Betula ermanii* or other Betulaceae, insert lime, rice bran in this, adjust water and you stuff blend which it is possible to canister or sack of the heat resistance, thermal sterilization do this and make sawdust culture medium.



this sawdust culture medium growth temperature, ideally of *Fuscoporia obliqua* mycelia after cooling 20°C or less, inoculation doing *Fuscoporia obliqua* pure mycelia in this culture medium, you grow *Fuscoporia obliqua* mycelia.

Until those of sclerotium (black) is possible to long period, ideal, after growing, it harvests, it extracts active ingredient with necessary treatment, it utilizes as antibacterial agent or foodstuff.

[0008]

active ingredient of *Fuscoporia obliqua* is extracted, there is a various methods, but for example natural *Fuscoporia obliqua* or mycelia of culture *Fuscoporia obliqua* is treated with PBS solution, butanol, ethanol, ethyl acetate or acetone, it is possible to remove active ingredient from each insoluble matter.

Or, natural *Fuscoporia obliqua* or adsorption it does mycelia component or mycelia extract of the culture *Fuscoporia obliqua* with carbon or *Thea sinensis* L. (tea) call, it is possible also to remove active ingredient from each non- adsorbant.

In addition, natural *Fuscoporia obliqua* or culture *Fuscoporia obliqua* active ingredient can be acquired even with hot water extraction you boil (for example 60 min boiling) with water of various pH.

These extraction active ingredient when it is a stable substance which is heat resistance, acid resistance with the water solubility are recognized with this invention.

These extracted component, that way, or mixing to foodstuffs, oral uptake is possible.

[0009]

Regarding to this invention, antibacterial agent action of *Fuscoporia obliqua* extract following to the minimum growth-inhibiting concentration (MIC) measurement method (Japan Society of Chemotherapy) concerning various bacterium namely *E. coli* O-157, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, gas necrosis microbe which is shown in pathogenicity bacterium, especially below-mentioned Table 1, it was verified by testing.

Each strain, specified bacterial count (inoculation bacterial count of Table 1) inoculation it did other than gas necrosis microbe, in the culture medium for sensitivity disk, in addition inoculation doing in 0.1% thioglycolic acid sodium adding plain heart infusion agar culture medium, it tested gas necrosis microbe with above-mentioned measurement method.

[0010]

Regarding to this invention, when you express one example of formation method of the *Fuscoporia obliqua* extract which you use for antibiotic activity test as follows is.

First, you insert powder, for example 100g of *Fuscoporia obliqua* (natural product or artificial culture) which powder fragment is done in distilled water 1000 cc, hot water extraction you do with 100°C by 40 min it starts boiling.

Next, extract is filtered making use of gauze or filter paper, furthermore centrifugal separation is done.

Approximately 10 min it does centrifugal separation, with centrifugal separator of for example 3000 rpm.

Next, it filters again, lyophilizing does, it obtains [chaga] extract active ingredient which is made objective.

In this way, *Fuscoporia obliqua* extract which is acquired is recognized those which have antibacterial agent action with a certain molecular weight or more.

In those which for example hot water-extracted matter that way, fraction are still not done, it can recognize marked antibiotic activity in general above molecular weight 100,000 table, but when fraction it does, as for active fraction it could recognize activity which is strong in especially molecular weight 30,000 or greater with hot water extraction.

In wide range it can recognize activity to molecular weight 10,000-5,000,000, but when the molecular weight 10,000-1,500,000, compared to there is a antibiotic activity ideally in range 10,000.-100,000, it is recognized.

Result of this antibiotic activity test is shown in Table 1.

[0011]

[Table 1]

microbe type inoculation bacterial count minimum growth-inhibiting concentration of pathogenicity bacterium (ppm)

E. coli O-157 (EDL 931)  $2.7 \times 10^7$  10, 000

(E. coli)

K. pneumoniae (ATCC 4352)  $8.0 \times 10^6$  10, 000

(Klebsiella pneumoniae)

P. aeruginosa (IFO 13276)  $2.8 \times 10^6$  5, 000

(Pseudomonas aeruginosa)

S. aureus (IFO 12732)  $2.2 \times 10^7$  5, 000

(Staphylococcus aureus)

B. subtilis spore (ATCC 6633)  $1.4 \times 10^6$  100, 000

(Bacillus subtilis)

C. perfringes H2  $2.0 \times 10^6$  2, 600

(gas gangrene microbe)

[0012]

antibiotic activity in order to verify whether those which are peculiar to the *Fuscoporia obliqua*, or being a antibacterial agent action even in other mushroom, did following test.

mushroom of candidate, other than *Fuscoporia obliqua*, maitake {*Grifola frondosa* (Dicks: Fr) }, is *Lentinus edodes* (Henn.) Sing. (Shiitake mushroom) {*Lentinus edodes* (Berk) Sing. }.

At a time each 100 g taking raw material of these candidate mushroom, you insert in canister, in this 40 min you start boiling with  $100^\circ\text{C}$  including distilled water 1000 cc, extract put out.

When this extract is filtered making use of Toyo filter paper No. A5, extract test liquid 200 cc of each candidate acquired.

antimicrobial test was done making use of this extract, making use of culture medium for similar sensitivity disk to description above.

Result is shown in Table 2.

[0013]

[Table 2]

specimen microbe type		Connection inoculation bacterial count most minimum growth-inhibiting concentration
(1) E.coli O157(EDL 931)		2.7×107 10,000 ppm
(1) E. coli O157 (EDL 931)		2.7 X 10,710,000 ppm
(2) E. coli O157 (EDL 931)		1.0 X 106 not be able to obstruct
(3) E. coli O157 (EDL 931)		1.0 X 106 not be able to obstruct
2 specimen [maitake]1 specimen Fuscoporia obliqua hot water bamboo hot water-extracted matter sample		

3 specimen [shiitake]Sample [0014]			
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basis which possesses antibacterial agent action where *Fuscoporia obliqua* is not an other mushroom is not grasped at present clearly. one of bioactivity which obeys human body from bacterium is the immunostimulating action.

There is a lymphocyte and a macrophage in cell which participates in the immunity, LPS (lipopolysaccharide) has contributed to activation of macrophage.

In order to verify, which extent it contains effective substance *Fuscoporia obliqua* to which this inventor pays attention this way, because it tested, result is shown in Table 3.

test agent, other than *Fuscoporia obliqua*, used *Daucus carota* L. var. *sativa* DC. (carrot) extract, *Daucus carota* L. var. *sativa* DC. (carrot) leaf extract.

It manufactured test agent, *Fuscoporia obliqua*, *Daucus carota* L. var. *sativa* DC. (carrot) extract, *Daucus carota* L. var. *sativa* DC. (carrot) leaf extract respectively 500 mg precisely weighing, it made final concentration 100 mg/ml in these including injectable distilled water.

This after with 56°C heating extracting in 3 hours water bath, centrifugal separation it did took centrifugation supernatant, designated this as starting liquid of measurement test agent.

It did quantification method of LPS, with specific color quantification method (Seikagaku Corporation (DB 69-058-4057)) of *Limulus amoebocytelysate* assay which can measure LPS in specific.

[0015]

[Table 3]

Inspection body test agent		LPS content ( $\mu\text{g/g}$ )	
Fuscoporia obliqua extract			1 and 610
Daucus carota L. var. sativa DC. (carrot) extract			0. 85
Daucus carota L. var. sativa DC. (carrot) leaf extract			29. 5

[0016]

From thing above, extract of Fuscoporia obliqua, possessing effective antibacterial agent action pathogenicity bacterium, especially recently with respect to O-157 which becomes non-normally problem was ascertained with this invention.

oral uptake it does Fuscoporia obliqua extract of this invention, in form of powder or liquid for example aqueous solution as drug, extermination does toxic pathogenicity bacterium and can obstruct to growth.

In addition same extract uptake can also do by to foodstuffs mixing adds as health food.

In powder, solution or other shapes adding and mixing doing in soy sauce, Miso, salt or other flavoring, it can designate *Fuscoporia obliqua* extract of for example this invention, as food.

In addition pan, cake, ice cream, chocolate, frozen confection, cooked rice, candy, in confection of jelly, oleaster, etc., or in the udon noodle, cold cereal grain, spaghetti or other processed food, adding and mixing doing *Fuscoporia obliqua* powder or solution when producing, it can make health food.

In same way, it is possible to mix to meat product of sausage, hamburger, croquet, tempura, fish past cake, flavoring meat, etc., when producing and, beer, wine, distilled rice spirits or other alcohols or alcohol beverage and fruit juice, carbonated beverage etc of the garlic extract, tomato, ginseng, vegetable mix juice, apple, grape etc., or furthermore mixing to black tea, oolong tea, yanron tea or other fermentation tea and coffee, green tea, cocoa beverage etc when producing, or when drinking it is possible also to make the health beverage.

Above, regarding to this invention, "health food " or "antimicrobial foodstuff " with generic it does those which add *Fuscoporia obliqua* extract flavoring, confection, processed food and to meat product, beverage which were illustrated.

[0017]

artificial culturing of *Fuscoporia obliqua*, aforementioned way has already been completed with difference application, but, top it is bitter in powder of hot water-extracted matter of especially sawdust culture among those, fragrance it does and dislikes and because there is a characteristic, it adds bitterness and the body to other than growth inhibition of pathogenicity bacterium, this beer and in addition by adding to beverage, In addition also application which is made substitute of hop as bitterness starting material is developed.

In addition, it is possible also *Fuscoporia obliqua* by fact that peculiar black pigment is utilized, to be possible, to replace to addition of the caramel at time of soy sauce production, to use *Fuscoporia obliqua* black powder in addition as the food coloring which is suited for safety and health retention.

black bun which had health carrying activity making use of black powder of for example *Fuscoporia obliqua* is made when, depth of color is put out to *Phaseolus angularis* L. (adzuki bean) bean jam when, in addition because there is not a toxic effect such as what on the health of human body, or it can apply to also white hair dyeing as the dye of fabric.

Furthermore, extract or extract of *Fuscoporia obliqua* at time of tobacco production by mixing to tobacco leaf, smoker without excessiveness uptake antimicrobial substance it is possible also that to try it is possible.

[0018]

[Effects of the Invention]

As in or more explanation, according to this invention extract of *Fuscoporia obliqua*, extermination doing fearful pathogenicity bacterium O-157 where directly as antibacterial agent the pathogenicity bacterium, especially time abnormal spread is shown closely is done by the regular uptake doing health food or antimicrobial foodstuff which by oral administration doing, or same extract adding and mixing, or action which growth inhibition is done is expected.

Because as for *Fuscoporia obliqua* extract of this invention, nontoxicity for human body with research of long year is verified, continuous administration doing, there are not times when it causes problem on health, there is not either a possibility of occurrence of resistant fungi like antibiotic which is used until recently.

health is kept by using antibacterial agent or antimicrobial foodstuff of this invention not just O-157, with respect to various disease which originates in pathogenicity bacterium, usual, widely, prevention is done work large practical effect which is acquired bacterial disease.